PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference VTT 138 PCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/FI2004/000439	International filing date *(day/month/year)* 08.07.2004	Priority date *(day/month/year)* 09.07.2003
International Patent Classification (IPC) or national classification and IPC C07K1/107		
Applicant VALTION TEKNILLINEN TUTKIMUSKESKUS et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09.05.2005	22.11.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Vanmontfort, D Telephone No. +49 89 2399-8457

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/FI2004/000439

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-23	as originally filed

Sequence listings part of the description, Pages

1-8	as originally filed

Claims, Numbers

1-11	filed with telefax on 30.09.2005

Drawings, Sheets

1/14-14/14	as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-11
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (SEPARATE SHEET)

International application No.
PCT/FI2004/000439

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

   D1: HUMPHREYS D.P. ET AL: 'Improved efficiency of site-specific copper(II) ion-catalysed protein cleavage effected by mutagenesis of cleavage site' PROTEIN ENGINEERING vol. 13, no. 3, 2000, pages 201 - 206, XP002903828

   D2: ALLEN G. ET AL: 'Specific cleavage of histidine-containing peptides by copper(II)' INT. J. PEPTIDE PROTEIN RES. vol. 48, 1996, pages 265 - 273, XP000623834

2. The subject-matter of claims 1-11 is novel and inventive (Articles 33(2) & 33(3) PCT). D2 (Table 1; abstract; page 272, column 2, lines 29-37), which is considered to represent the closest prior art, discloses the use of Cu2+ ions for cleavage of histidine-containing peptides. The best results were obtained in peptides containing Ser-His or Thr-His sequences.
   The subject-matter of claim 1 differs from this in that the amino acid sequence comprises XiXi, or repeats thereof or two or more repeats of XiYn, wherein n=l, Xi is His and Yn is any amino acid.

   The subject-matter of claim 1 is therefore new (Article 33(2) PCT).

   The problem to be solved by the present invention may be regarded as the provision of alternative cleavage site for cleaving proteins.

   The solution to this problem proposed in claim 1 of the present application is considered as involving an inventive step (Article 33(3) PCT) for the following reasons:
   There is no indication in any of the available prior art documents of using pairs of histidine (His-His) or repeats of pairs in which histidine is the first amino acid. The preferred sequences of D2 have histidine on the second spot (Ser-His or Thr-His). D1 discloses 5 oligopeptides (Table 1), in which histidine is on the last spot.

There is no guidance in any of the available prior art documents for the skilled person to construct the predetermined cleavage sites as claimed in claim 1. Moreover, the sequences of the present invention have the advantage to allow cleavage at broader pH range, lower temperatures and shorter reaction times.

Claims 2-11 are dependent on claim 1 and as such also meets the requirements of the PCT with respect to novelty and inventive step.

CLAIMS

1. A method for cleaving a protein or peptide at a specific site, characterized in that the method comprises the steps:
- constructing at a predetermined cleavage site of the protein or peptide an amino acid sequence of 2 to 20 amino acids, wherein the amino acid sequence comprises X_1 X_1 or repeats thereof or two or more repeats of X_1Y_n, wherein n=1, X_1 is His and Y_n is any amino acid, and
said amino acid sequence is cleavable in the presence of free metal ions,
said amino acid sequence does not exist naturally in the protein or peptide to be cleaved; and
- allowing said protein or peptide to react with the metal ion in a buffer, said buffer further comprising a reducing or oxidizing agent or agents.

2. The method according to claim 2, wherein the length of the amino acid sequence is 2 to 10 amino acids, preferably 4 to 8 amino acids.

3. The method according to claim 2 or 3, wherein the amino acid Y_n is selected from the group comprising Cys, Lys and Trp.

4. The method according to any one of the preceding claims, wherein the amino acid Y_n is His or Lys.

5. The method according to any one of the preceding claims, wherein the amino acid sequence comprises a sequence selected from the group

$(His)_2$, $(His)_4$ (SEQ ID NO:28), $(His)_6$ (SEQ ID NO:29), $(His)_8$ (SEQ ID NO:30) and His-Ser-His-Ala-His-Gly-His-Ala-His-Ser-His-Gly (SEQ ID NO:9).

6. The method according to any one of the preceding claims, wherein the metal ion is a ion of a metal selected from the group of transition metals, preferably from the group comprising Cu, Co, Ni, Fe, Mn, Cd, Pd, Rh, Ru, Pt, Cr and Zn.

7. The method according to any one of the preceding claims, wherein the metal ion is a ion of a metal selected from the group comprising Cu, Co, Mn, Cr, Ni , Fe and Zn, preferably from the group comprising Cu and Co.

8. The method according to any one of the preceding claims, wherein the metal ion is a ion of Cu.

9. The method according to any one of the preceding claims, wherein the protein to be cleaved is a recombinant protein.

10. The method according to any one of the preceding claims, wherein the amino acid sequence is constructed at a predetermined cleavage site by genetic engineering methods.

11. The method according to any one of the preceding claims, wherein the reaction is carried out in the presence of a reagent selected from the group comprising hydrogen peroxide, ascorbate and dithiothreitol or in the presence of a combination of these reagents.

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